Leveraging ageing models of pulmonary fibrosis: the efficacy of nintedanib in ageing

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To the Editor:

Nintedanib is one of two US Food and Drug Administration (FDA)-approved treatments for idiopathic pulmonary fibrosis (IPF). The clinical efficacy of nintedanib for inhibiting the progression of lung fibrosis is well-established [1]. However, although nintedanib is overwhelmingly prescribed to elderly patients, the impact of ageing on its efficacy is difficult to discern from clinical data due to the magnitude of confounding variables that exist among human subjects (genetics, gender, comorbidities, disease stage at the onset of treatment, *etc*.). A recent *post hoc* meta-analysis of five IPF clinical trials suggested that the effect of nintedanib in reducing the rate of forced vital capacity decline is consistent across patients with age (patients >75 *versus* patients <75 years of age) [2]. However, it is important to note that the average age of IPF diagnosis is 66 years and the average patient ages in these cohorts were 78 (>75) *versus* 64 (<75) years. Further, one could argue that patients in both cohorts represent the elderly population. This study highlights the complexity of evaluating the impact of ageing on efficacy in a clinical setting. To date, all pre-clinical efficacy studies with nintedanib have been performed in young animals. We therefore sought to determine whether ageing impacts the efficacy of nintedanib for inhibiting the development of lung fibrosis. Bleomycin-induced lung injury in young (2 month) and aged (18 month) mice was followed by treatment with nintedanib or vehicle from day 10–21 (figure 1a), using a previously described protocol [3]. We previously demonstrated in this injury model that the severity of lung fibrosis is identical in young and aged mice, in terms of the net increase in total lung collagen following injury [4]. Although some prior studies have reported seemingly contradictory results, indicating increased severity of fibrosis in aged mice [5, 6], this discrepancy could be attributed to increased baseline levels of collagen in aged mice and the methodology/analyses used for fibrosis assessment, as the net increase in collagen appear to be similar in both young and aged mice [5, 6]. In line with our previous findings, both young and aged vehicle-treated mice demonstrated similar levels of fibrosis severity and a similar decline in lung function at 3 weeks post-injury (figure 1b–d, g–h). Also consistent with numerous prior reports [7, 8], we found that in young mice, nintedanib demonstrated efficacy for inhibiting the development of fibrosis (figure 1b–g) and led to improved lung function (figure 1h). Interestingly, nintedanib also significantly inhibited the development of lung fibrosis in aged mice, to a similar extent as young cohorts (figure 1b–g). Although nintedanib treatment resulted in lung functional improvement to a similar extent in both young (49%) and aged (57%) mice (figure 1h), results did not reach statistical significance in aged mice. Of note, there is less than 47% power to detect mean differences between the aged-vehicle and aged-nintedanib groups given the observed effect and sample sizes of aged mice; the trending p-value of 0.06 is displayed to provide a better understanding of the results. No significant differences in survival rate were observed between nintedanib- *versus* vehicle-treated groups for both young (68% *versus* 72%, respectively) and aged mice (83% *versus* 76%, respectively) during this treatment period (day 10–21). Overall, these data indicate that ageing does not impact the efficacy of nintedanib in terms of its ability to inhibit the development of *de novo* lung fibrosis.

Most IPF patients exhibit progressive lung fibrosis. Although this bleomycin model is not typically considered to be a model of "progressive fibrosis", one could argue that the development of *de novo* fibrosis is a component of progressive fibrosis. This is the first study to suggest that age alone does not impact the efficacy of nintedanib for inhibiting the development of *de novo* lung fibrosis. This is reassuring from a clinical standpoint, given that nintedanib is predominantly administered to elderly patients [1].

Shareable abstract (@ERSpublications)
Although nintedanib is overwhelmingly prescribed to elderly patients, this is the first study to demonstrate that ageing does not impact the efficacy of nintedanib. This study sheds light on the utility of aged animal models in pulmonary fibrosis. https://bit.ly/3zA9RC5

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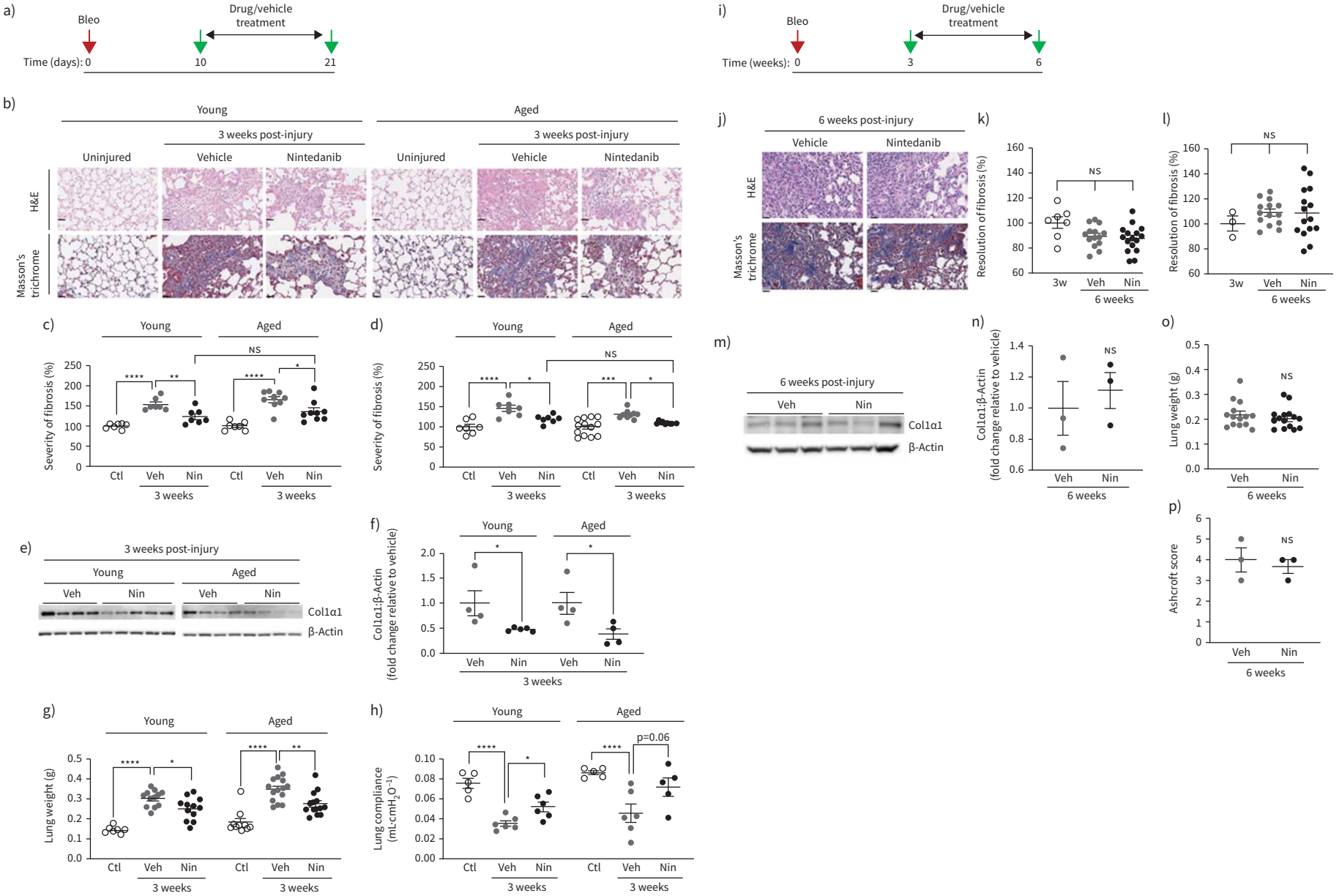

FIGURE 1 Nintedanib inhibits pulmonary fibrosis development in aged mice but does not reverse age-dependent established fibrosis. a–h) C57BL/6 young (2 month) and aged (18 month) male mice received intratracheal instillation of bleomycin (0.02875 U per mouse) were treated with nintedanib (60 mg·kg^{-1} dissolved in deionised water) or vehicle (deionised water) *via* oral gavage daily from days 10–21. All mice were evaluated at 21 days post-injury. a) Schematic diagram illustrating treatment protocol. b) Lung tissue was assessed by haematoxylin and eosin (H&E) staining for histopathology and Masson's trichrome staining for collagen. c) Total lung collagen was determined by quantitative Sircol assay. Data are presented as % change at 3 weeks post-injury relative to respective uninjured control group. d) Total lung hydroxyproline was analysed by quantitative hydroxyproline assay. Data are presented as % change at 3 weeks post-injury relative to respective uninjured control group. Collagen-1α1 expression was assessed by e) Western blot and f) densitometric analysis. g) Lung weight at day 0 and day 21 (pre- and post-injury/treatment, respectively). h) Static lung compliance was determined by FlexiVent. i–p) Aged (18 month) C57BL/6 female mice were administered bleomycin (0.03125 U per mouse) *via* intratracheal instillation. Starting at 3 weeks post-injury (when aged mice demonstrate established/persistent fibrosis), nintedanib (60 mg·kg^{-1}) or vehicle was administered daily from 3–6 weeks *via* oral gavage. i) Schematic diagram illustrating treatment protocol. j) Lung tissue was assessed at 6 weeks post-injury by H&E staining for histopathology and Masson's trichrome staining for collagen. k) Total lung collagen at 6 weeks post-injury was determined by quantitative Sircol assay. Data are presented as % change at 6 weeks relative to 3 weeks post-injury. l) Total lung hydroxyproline was analysed by quantitative hydroxyproline assay. Data are presented as % change at 6 weeks relative to 3 weeks post-injury. Collagen-1α1 expression was assessed by m) Western blot and n) densitometric analysis. o) Lung weight at 6 weeks post-injury is shown. p) Ashcroft scoring was evaluated by HistoWiz (Brooklyn, NY, USA). All dot plot data are shown as mean±SEM. Two-tailed t-test was used for comparisons between two groups and one-way ANOVA was used to compare three or more groups. Bleo: bleomycin; Ctl: control; Veh: vehicle; Nin: nintedanib. *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001; NS: nonsignificant.

It is tempting to speculate that the efficacy of nintedanib in ageing (as described here) may have been a fortuitous feature that contributed to its success in clinical trials, particularly since numerous drug candidates demonstrating efficacy in young mice have failed in clinical trials with elderly patients. No studies have evaluated whether ageing impacts the efficacy of pirfenidone for inhibiting the development of fibrosis. However, if age-dependent differences in the efficacy of pirfenidone are identified, such findings could have clinical implications for the selection of therapeutics based on patient age.

It is well established that nintedanib inhibits progressive lung decline in IPF patients [1]. The prevailing understanding has been that nintedanib inhibits fibrosis progression. However, no studies have evaluated whether nintedanib can reverse established fibrosis. Such studies are not feasible in young mice, as young mice demonstrate a self-limited fibrotic response where fibrosis spontaneously resolves following peak injury [4, 9]. Conversely, aged mice exhibit a persistent fibrotic response, with little to no resolution of fibrosis at 4 months post-injury [4]. This ageing model of persistent lung fibrosis offers a more clinically relevant testing protocol, where the therapeutic efficacy of agents can be evaluated for their ability to resolve age-dependent persistent fibrosis; no studies have previously evaluated the efficacy of nintedanib on reversing age-dependent established fibrosis. We therefore administered nintedanib or vehicle to aged mice daily from 3–6 weeks post-injury (when aged mice demonstrate established/persistent fibrosis) (figure 1i) [4]. Like our previously published studies [4], we found that aged vehicle-treated mice exhibited persistent fibrosis during this time period (from 3–6 weeks) (figure 1j). However, nintedanib treatment failed to rapidly promote the resolution of age-dependent established lung fibrosis during the 3-week treatment period (figure 1j–p). Further, no significant survival benefit was observed between vehicle- and nintedanib-treated aged mice (73.9% *versus* 78.2%, respectively) during this treatment period (3–6 weeks). It is possible that increased duration of nintedanib treatment may be required in order to definitively evaluate the efficacy of nintedanib for reversing age-dependent established fibrosis. Senescent fibroblasts secrete elevated levels of platelet-derived growth factor (a target of nintedanib) [10], and aged mice exhibit a persistent senescence response associated with non-resolving fibrosis for up to 4 months post-injury [4]. To date, no therapy has demonstrated the ability to reverse age-associated established fibrosis, which may represent the holy grail for therapeutic strategies to more effectively treat and/or cure IPF.

Although the FDA-approval of two therapeutic agents represents a significant breakthrough by providing the first available treatment options for IPF patients, these therapies do not cure IPF or significantly improve the quality of life for IPF patients. Thus, the search for more effective therapies continues. One possible explanation for the limited therapies available is that age-dependent pathologic mechanisms remain largely unexploited in the drug development process, despite the fact that ageing has been strongly implicated in the pathogenesis of IPF [11, 12]. Numerous studies have demonstrated the presence of premature ageing hallmarks, including the accumulation of senescent cells, in the lungs of IPF patients [4, 13]. However, senescence can be pro- or anti-fibrotic, depending on the context of age [4, 14]. Following injury in young mice, senescent cells undergo apoptosis, which promotes fibrosis resolution [4, 14]. However, in aged mice, senescence leads to the acquisition of an apoptosis-resistant myofibroblast phenotype, which promotes fibrosis persistence [4]. With the emergence of senolytics

(senescence-targeting therapeutic agents) as a promising treatment strategy for IPF [15], age-relevant pre-clinical efficacy models will be critical to accurately evaluate their potential. Key opinion leaders recommend that ageing models should be employed when evaluating age-related targets [16–18], as testing of senolytics in young animal models alone may not provide a fully comprehensive assessment of their potential. Overall, this study sheds light on the potential utility of aged animal models, which provide opportunities to identify/validate age-dependent pathologic mechanisms, address specific research questions, and/or employ more rigorous efficacy testing protocols for evaluating therapeutic agents. Incorporating ageing models at the appropriate stages of research and/or therapeutic development is likely to provide novel insight that would accelerate the successful translation of improved therapies for IPF, a disease that disproportionately afflicts the elderly population.

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